INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 02/05846

Box I Observations where certain claims were f und unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-6 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 1-4
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-4

Present claims 1-4 relate to an extremely large number of possible compounds. Support within the meaning of Art. 6 PCT and/or disclosure within the meaning of Art. 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds disclosed in claim 5, as well as NO-Ketorolac (cf. Example F1).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 02/05846

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Published:
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/092072 A2

(54) Title: DRUGS FOR THE ALZHEIMER DISEASE

(57) Abstract: Use for the treatment of the Alzheimer disease of compounds or salts thereof, having the following general formula (I): A-$(B)_{b0}$-$(C)_{c0}$-NO_2, wherein A contains the radical of a drug having an antiinflammatory activity, B is a bivalent linking group the precursor thereof must satisfy the tests described in the application, C is a linking group as defined in the invention.

DRUGS FOR THE ALZHEIMER DISEASE

* * * * *

The present invention relates to the use of a particular class of drug nitrooxyderivatives in the prophylaxis and therapy of the Alzheimer disease.

The Alzheimer is a disease which mainly affects old people. The progressive ageing of people determines a significant increase of the incidence of said disease: at present in the industrialized countries the people affected by the Alzheimer disease are about 13.5 millions.

The initial symptoms of the Alzheimer dementia include a decrease of the cognitive faculties. The main characteristics of the disease comprise the following:

- amyloid deposits (amyloid plaques) in the brain parenchyma and in the vessel wall, the main component of said plaques is the ß-amyloid protein (Aß);
- inflammatory process characterized by the activation of the microglia and by the presence of reactive astrocytes in the peri-plaque area with production of pro-inflammatory mediators, such as cytokines, NO.

Recently it has been observed that non steroidal inflammatory drugs (NSAIDs) can be useful in the disease prophylaxis and therapy (Stewart WF, Neurology 48, 626-632, 1997). These compounds have a good antiinflammatory activity at brain level, however they have the drawback not to have a high inhibition of the amyloid plaque deposition. Therefore these compounds have a limited efficacy in preventing or in the treatment of the Alzheimer disease.

Another therapeutic approach for the prevention and the treatment of the Alzheimer disease is the administration of

beta-amyloid vaccines (Schenk D. et Al. Nature 400 (6740) 173-7, 1999) or antibodies against amyloid β-peptide (Aβ) (F. Bard et Al. Nature vol. 6, 8, 916-919 2000). The vaccine action mechanism seems to be due to the antibody reaction versus the beta-amyloid protein. The vaccine activity in the prevention, reduction and in the treatment of the amyloid plaque deposit is higher with respect to the antiinflammatory drugs. However vaccines show the drawback to have a high immunotoxicity due to the antibody hyperproduction (Schenk, see above).

The need was felt to have available more effective compounds in the prevention, reduction and in the treatment of the deposit of the amyloid plaque with respect to the drugs of the prior art.

An object of the present invention is the use in the prophylaxis and treatment of the Alzheimer disease of compounds or salts thereof having the following general formula:

$$A\text{-}(B)_{b0}\text{-}(C)_{c0}\text{-}NO_2 \qquad (I)$$

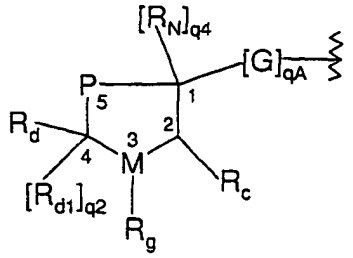
wherein:

c0 is an integer and is 0 or 1, preferably 1;

b0 is an integer and is 0 or 1, with the proviso that c0 and b0 cannot be contemporaneously equal to zero.

A = R-T_1-, wherein

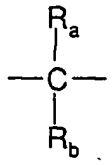
R is the drug radical as defined below:



(IV)

wherein:

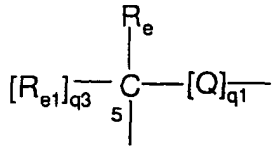
G is one of the following groups: -CH=CH-



(Vg)

M is one carbon or nitrogen atom;

P is the following group:



(V)

wherein:

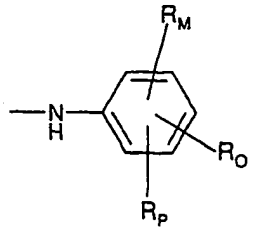
the carbon atom of formula (V) is in position 5 of the ring of formula (IV);

Q is -CH- or one oxygen atom;

q_A, q_1, q_2, q_3, q_4, are integers and independently the one from the other are 1 or 0; $q_2 = q_3 = q_4 = 0$ when the ring in formula (IV) is aromatic and Q = -CH-; $q_2 = q_3 = q_4 = 1$ when the ring of formula (IV) is a saturated ring with 6 atoms wherein the heteroatom is Q = O, and is in position 6 of the ring;

when q_A = 1 and G is the group of formula (Vg) R_a and R_b, equal or different, are hydrogen, C_1-C_3 alkyl, preferably methyl;

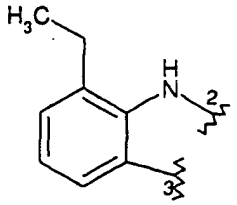
R_c is hydrogen, C_1-C_3 alkyl, or the following radical:



(VI)

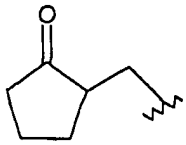
wherein R_M, R_O, R_P, equal or different, can be H, halogen preferably chlorine, C_1-C_3 alkyl, preferably methyl, CF_3;

R_g is hydrogen or OCH_3 when the ring of fig. (IV) contains 6 atoms; or it is an electronic doublet when the ring with 6 atoms is aromatic and M = nitrogen; or it is a p.chlorobenzoyl radical when q_1 = 0 and M = nitrogen and the ring of fig. (IV) is aromatic; or R_c and R_g taken together are such to form the following radical:



(VII)

R_d in formula (IV) is hydrogen, hydroxyl, C_1-C_4 alkyl, optionally branched, phenyl, or the following radical:

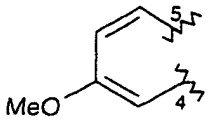


(VIII)

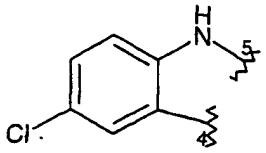
R_{d1} = hydrogen when q_2 = 1;

R_e = hydrogen, halogen preferably F, or benzoyl; or

R_d and R_e taken together are such to form the following radicals:



(IX)



(X)

R_{e1} = H when q_3 = 1;

R_N = C_1-C_3 alkyl preferably ethyl when q_4 = 1;

T_1 = $(CO)_t$ or $(X)_{t'}$, wherein X = O, S, NR_{1c}, R_{1c} is H or a linear or branched alkyl, having from 1 to 5 carbon atoms, t and t' are integers and equal to zero or 1, with the proviso that t = 1 when t' = 0; t = 0 when t' = 1;

B = $-T_B-X_2-T_{BI}-$ wherein

T_B and T_{BI} are equal or different;

T_B = (CO) when the reactive function in the precursor drug is -OH or $-NH_2$; T_B = X, as above, when the reactive function in the precursor drug is -COOH;

T_{BI} = $(CO)_{tx}$ or $(X)_{txx}$, wherein tx and txx have the value of 0 or 1; with the proviso that tx = 1 when txx = 0, tx = 0 when txx = 1; X is as above defined;

X_2 is a bivalent linking group as below defined;

C is the bivalent radical $-T_c-Y-$ wherein:

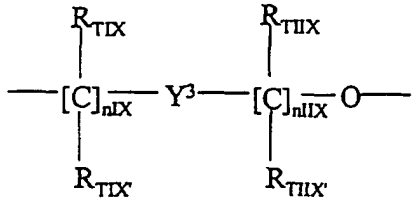
when b0 = c0 = 1: T_c = (CO) when tx = 0, T_c = X when txx = 0, X being as above defined,

when b0 = 0: T_c = (CO) when t = 0, T_c = X when t' = 0, X being as above defined,

when c0 = 0: tx = 0, T_{BI} = X = –O–;

Y has one of the following meanings:

Y_p :



(III)

wherein:

nIX is an integer from 0 to 3 , preferably 1;

nIIX is an integer from 1 to 3 preferably 1;

R_{TIX}, $R_{TIX'}$, R_{TIIX}, $R_{TIIX'}$, equal to or different from each other are H or linear or branched C_1-C_4 alkyl; preferably R_{TIX}, $R_{TIX'}$, R_{TIIX}, $R_{TIIX'}$ are H;

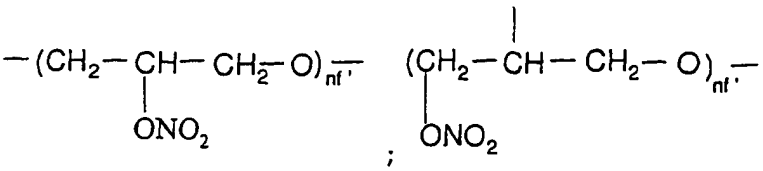
Y^3 is a heterocyclic ring containing one or two nitrogen atoms, said heterocyclic ring being a saturated, unsaturated or aromatic ring, having 5 or 6 atoms;

or Y can be:

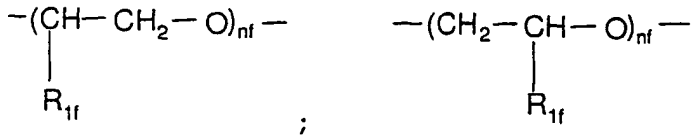
Y_0, selected from the following:

- an alkylenoxy group R'O wherein R' is a linear or branched when possible C_1-C_{20}, preferably having from 2 to 6 carbon atoms, or a cycloalkylene having from 5 to 7 carbon atoms, in the cycloalkylene ring one or more carbon atoms can be substituted with heteroatoms, the ring can have side chains of R' type, R' being as above;

or one of the following groups:

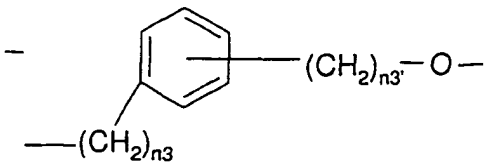
- 

wherein nf' is an integer from 1 to 6 preferably from 1 to 3;

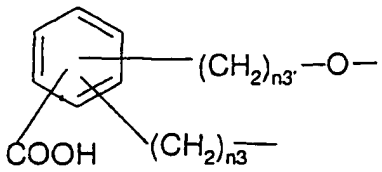
- 

wherein R_{1f} = H, CH_3 and nf is an integer from 1 to 6; preferably from 2 to 4;

or Y is Y_{Ar} and is selected from the following:

- 

wherein n3 is an integer from 0 to 3 and n3' is an integer from 1 to 3;

- 

wherein n3 and n3' have the above meaning;

with the proviso that in formula (I) when b0 = 0 and the bivalent radical Y of C is R'O, the radical R of formula (IV) of the drug is ferulic acid or flurbiprofen;

- X_2, bivalent radical, is such that the corresponding precursor of B -T_B-X_2-T_{BI}- wherein the free valences of T_B and T_{BI} are each saturated with OZ, with Z or with -N(Z^I)(Z^{II}), being:

- Z = H, C_1-C_{10}, preferably C_1-C_5 alkyl linear or branched when possible,
- Z^I, Z^{II} equal or different have the Z values as above, depending on that T_B and/or T_{BI} = CO or X, in function of the values of t, t', tx and txx;

it satisfies the following test (test 4): analytical determination carried out by adding aliquots of methanolic solutions at 10^{-4} M concentration of the precursor of B to a methanolic solution of DPPH (2,2-diphenyl-1-picryl hydrazyl); after having maintained the solution at room temperature and sheltered from light for 30 minutes, the absorbance of the test solution and of a solution containing only DPPH in the same amount is read, at the wave length of 517 nm; then the inhibition percentage of the precursor of B towards the radical production induced by DPPH is determined by means of the formula:

$$(1 - A_s/A_c)x100$$

wherein A_s and A_c are respectively the absorbance values of the solution containing the test compound and DPPH and that of the solution containing only DPPH.

The acceptance criterion of the precursor compounds of B according to this test is the following: test 4 is satisfied by the precursor compounds of B when the inhibition percentage as above defined is higher than or equal to 50%.

When in formula (IV) q_A = 1 and G is the group of formula (Vg) wherein R_a is methyl and R_b is hydrogen, q_2 = q_4 = 0, M = C, and in formula (V) q_1 = 1, Q = -CH- and q_3 = 0, the ring of formula (IV) comprising M and Q is an aromatic ring having 6 carbon atoms and the other substituents are as defined hereinafter:

- when R_c = R_g = R_e= H and R_d is isobutyl, the so defined precursor drug of R is ibuprofen;
- when R_c = R_g = H and R_d is phenyl and R_e is F, the so defined precursor drug of R is flurbiprofen;
- when R_c = R_g = H and R_d and R_e form together the radical of formula (IX), the so defined precursor drug of R is naproxen;
- when R_c = R_g = R_e = H and R_d is the radical of formula (VIII), the so defined precursor drug of R is loxoprofen;
- when R_c = R_g = R_d = H and R_e = benzoyl, the so defined precursor drug of R is ketoprofen;
- when R_c = R_g = H and R_d and R_e form together the radical of formula (X), the so defined precursor drug of R is carprofen;

when in formula (IV) q_A = 0, q_2 = q_4 = 0, R_d = R_g = H, M = C, and in formula (V) q_1 = 1, Q = -CH-, q_3 = 0, R_e = H, the ring of formula (IV) comprising M and Q is an aromatic ring having 6 carbon atoms and the other substituents are defined as hereinafter:

- when R_c is the radical of formula (VI) wherein R_M = R_P = H, R_O = CF_3 and is in meta position with respect to the group -NH-, the so defined precursor drug of R is the flufenamic acid;
- when R_c is the radical of formula (VI) wherein R_M = R_P = Cl and are in the two orto positions with respect to the -NH- group, R_O = CH_3 and is in para position with respect to the -NH- group, the so defined precursor drug of R is the meclofenamic acid;
- when R_c is the radical of formula (VI) wherein R_M = H, R_P = Cl and is in meta position with respect to the -NH-

group, R_O = CH_3 in orto position with respect to the -NH- group and to the chlorine atom, the so defined precursor drug of R is the tolfenamic acid;

when in formula (IV) q_A = 0, M = N; q_2 = q_4 = 0, R_d = H; and in formula (V) q_1 = 1, q_3 = 0, R_e = H, Q = -CH-; R_g is the free electronic doublet on the nitrogen atom, the ring of formula (IV) comprising M and Q is a pyridine ring, R_c is the radical of formula (VI) wherein R_M = R_P = H, R_O = CF_3 and is in meta position with respect to the -NH- group, the so defined precursor drug of R is the niflumic acid;

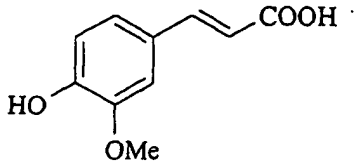
when in formula (IV) q_A = 1 and G is the group of formula (Vg) wherein R_a = R_b = H; M = C, R_d = R_g = H, q_2 = q_4 = 0; and in formula (V) q_1 = 1, Q = -CH-, R_e = H, q_3 = 0; the ring of formula (IV) comprising M and Q is an aroamtic ring having 6 carbon atoms; R_c is the radical of formula (VI) wherein R_M = R_P = Cl and are in the two ortho positions with respect to the -NH- group, R_O = H; the so defined precursor drug of R is diclofenac;

when in formula (IV) q_A = 1 and G is the group of formula (Vg) wherein R_a = R_b = H; M = C, q_2 = q_4 = 1, R_d = R_{d1} = H, R_N = ethyl, and in formula (V) q_1 = 1, q_3 = 1, Q = O, R_e = R_{e1} = H; the ring of formula (IV) comprising M and Q is a saturated ring having 6 atoms; R_g and R_c together form the radical of formula (VII), the so defined precursor drug of radical R is etodolac;

when in formula (IV) q_A = 1 and G is the group of formula (Vg) wherein R_a = R_b = H; M = N q_2 = q_4 = 0; and in formula (V) q_3 = q_1 = 0, the ring in formula (IV) comprising M corresponds to that of pyrrole; R_g = p.chlorobenzoyl; R_c = CH_3; R_d together with R_e of formula (V) form the radical of

formula (IX), the so defined precursor drug of radical R is indomethacin;

when in formula (IV) q_1 = 1 and G = -HC=CH-, q_2 = q_4 = 0, M = C, and in formula (V) q_1 = 1, Q = -CH-, q_3 = 0 and R_e = H, the ring of formula (IV) comprising M and Q is an aromatic ring having 6 carbon atoms; R_c = H, R_g = OCH_3, R_d = OH, the so defined precursor drug of radical R is the ferulic acid of formula (IVA)



(IVA)

The precursor compound of B which satisfies test 4 is preferably selected from the following classes of compounds:

- aminoacids, selected from the following:: L-carnosine, anserine, selenocysteine, selenomethionine, penicillamine, N-acetylpenicillamine, cysteine, N-acetylcysteine, glutathione or esters thereof, preferably ethyl or isopropyl ester;
- hydroxyacids, selected from the following: gallic acid, ferulic acid, gentisic acid, citric acid, caffeic, dihydrocaffeic acid, p-cumaric acid, vanillic acid;
- aromatic and heterocyclic polyalcohols, selected from the following: nordihydroguaiaretic acid, quercetin, catechin, kaempferol, sulfuretine, ascorbic acid, isoascorbic acid, hydroquinone, gossypol, reductic acid, methoxyhydroquinone, hydroxyhydroquinone, propyl gallate, saccharose, 3,5-di-tertbutyl-4-hydroxybenzyl-thio glycolate, p-cumaric alcohol, 4-hydroxy-phenyl-ethylalcohol, coniferyl alcohol, allopurinol;

- compounds containing at least one free acid function, selected from the following: 3,3'-thiodipropionic acid, fumaric acid, dihydroxymaleic acid, edetic acid.

The precursor compounds of B of the above mentioned groups are prepared according to methods known in the prior art and described, for example, in "The Merck Index", 12a Ed. (1996), herein incorporated by reference.

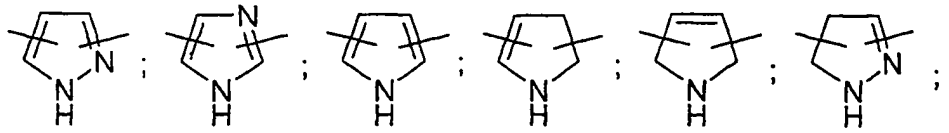
Test 4 is a colorimetric test which allows to establish whether the precursors of B are able to inhibit the production of radicals from DPPH (2,2-diphenyl-1-picryl-hydrazyl) (M.S. Nenseter et Al., Atheroscler. Thromb. 15, 1338-1344, 1995). 100 μM solutions in methanol of the tested substances are prepared, and an aliquot of each of said solutions is added to a 0,1 M DPPH solution in methanol. After having stored the solutions at room temperature and sheltered from light for 30 minutes, the absorbance is read at the wave length of 517 nm. The absorbance decrease with respect to that of the solution containing the same DPPH concentration is determined. The effectiveness of the tested compound in inhibiting the production of radicals is expressed by the following formula:

$$(1 - A_s/A_c)x100$$

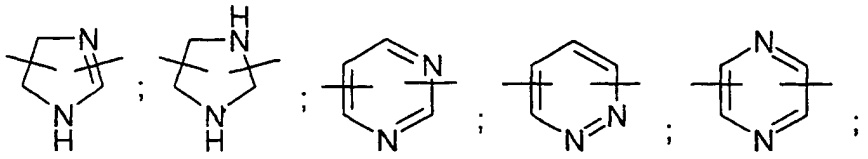
wherein A_s and A_c are respectively the absorbance values of the solution containing the tested compound together with DPPH and of the solution containing only DPPH.

The precursor of B satisfies test 4 if its effectiveness in inhibiting the radical production, as above defined, is equal to or higher than 50% at the indicated concentration (10^{-4} M).

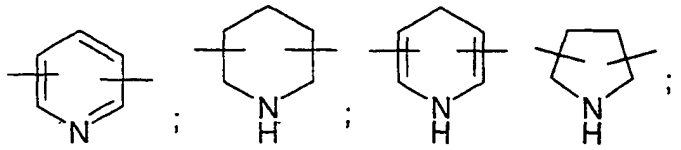
Preferably Y^3 is selected from the following:



(Y1) (Y2) (Y3) (Y4) (Y5) (Y6)



(Y7) (Y8) (Y9) (Y10) (Y11)



(Y12) (Y13) (Y14) (Y15)

Preferably Y^3 is an aromatic ring having 6 atoms, containing one nitrogen atom, said aromatic ring having the two free valences in position 2 and 6.

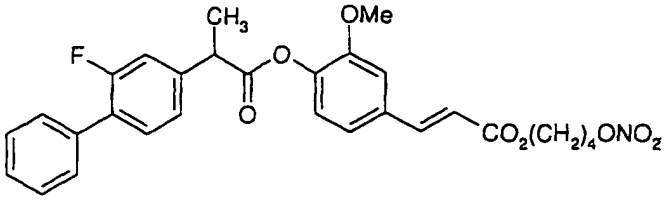
The preferred of Y^3 is Y12 (pyridyl) susbstituted in position 2 and 6. The bonds can also be in asymmetric position. For example Y12 (pyridyl) can be substituted also in position 2 and 3; Y1 (pyrazol) can be 3,5-disubstituted.

The precursors of Y as defined by formula (III) wherein the free valence of the oxygen is saturatd with H and the free valence of the end carbon is saturated either with a carboxylic or hydroxylic group, are products available on the market or can be obtained by methods known in the prior art.

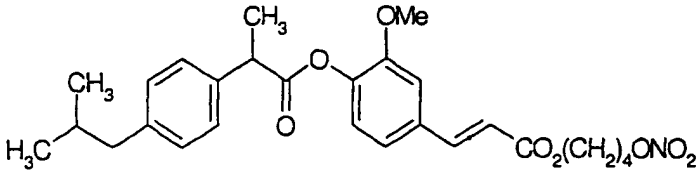
In formula (I) the preferred precursors of B for the synthesis of the nitrooxyderivatives usable in the present invention are the following: ferulic acid, N,acetylcysteine, cysteine, caffeic acid, hydrocaffeic and gentisic acid; the preferred precursor drugs of R are the following: ibuprofen, flurbiprofen, naproxen, ferulic acid of formula (IVA).

The preferred compounds of formula (I) for the use according to the present invention are the following:

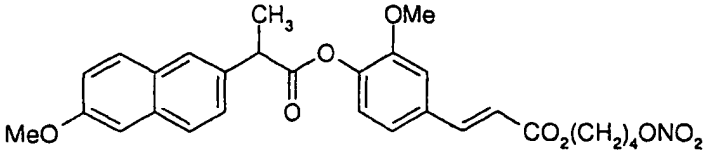
- [1,1'-biphenyl]-4-acetic acid-, 2-fluoro-alpha-methyl-, 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxo-1-propenyl]phenylester (XII);
- alpha-methyl-4-(2-methylpropyl)benzenacetic acid-, 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxo-1-propenyl]phenylester (XIII);
- 6-methoxy-alpha-methyl-2-naphthalenacetic acid-, 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxo-1-propenyl] phenylester (XIV);
- (S)-N-acetylcysteine-4-(nitrooxy)butylester-,(S)-6-methoxy-alpha-methyl-2-naphthalenacetate (XV);
- (S)-N-acetylcysteine-4-(nitrooxy)butylester-, 2-fluoro-alpha-methyl-[1,1-biphenyl]-4-acetate (XVI);
- (S)-N-acetylcysteine-4-(nitrooxy)butylester-, 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-acetate (XVII);
- (S)-N-acetylcysteine-4-(nitrooxy)butylester-, alpha-methyl-4-(2-methylpropyl)benzeneacetate (XVIII);
- 2-[(2,6-dichlorophenyl)amino]benzeneacetic acid-, 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxo-1-propenyl] phenylester (XIX);
- (S)-N-acetylcysteine-4-(nitrooxy)butylester-, 2-[(2,6-dichlorophenyl)amino]benzeneacetate (XX);



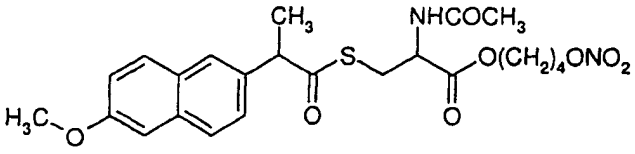
(XII)



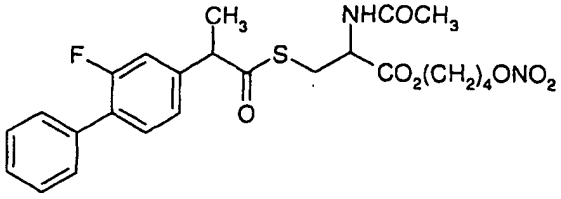
(XIII)



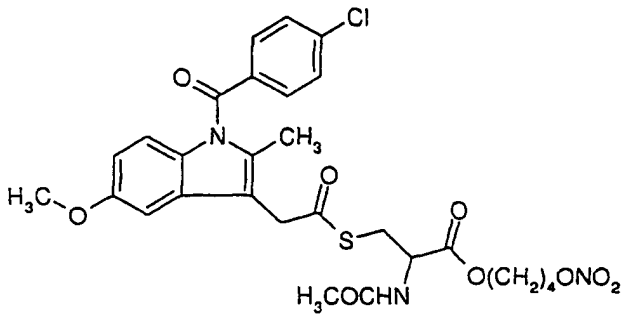
(XIV)



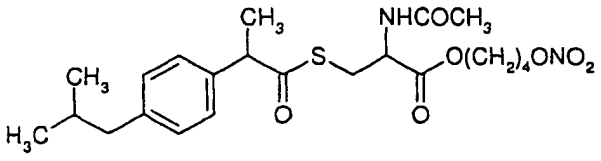
(XV)



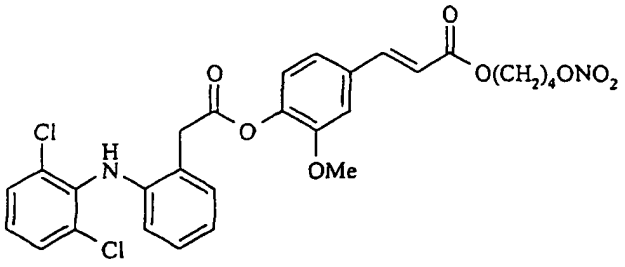
(XVI)



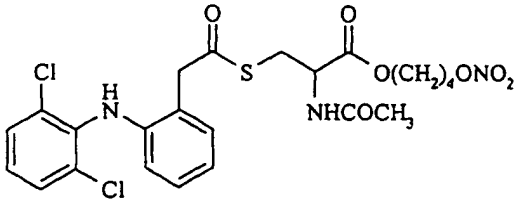
(XVII)



(XVIII)



(XIX)

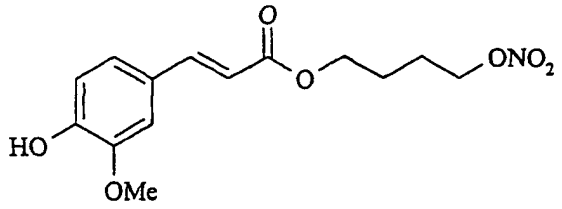


(XX)

Other preferred compounds of the invention are those wherein in formula (I) the precursor drug of R has formula

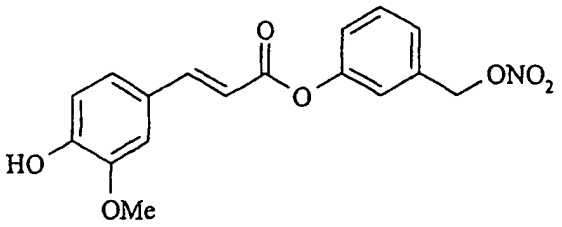
(IVA), b0= 0 and c0 = 1 and C is Y_0 or Y_{Ar} or Y_p, in particular the following compounds can be mentioned:

- trans 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid-, 4-(nitrooxy)butyl ester (XXI)



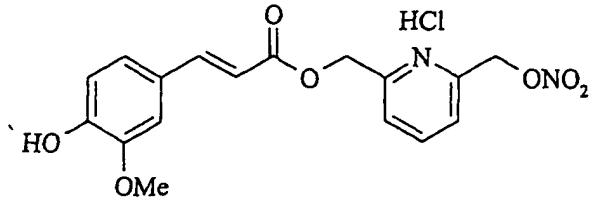
(XXI)

- trans 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid-, 3-(nitrooxymethyl)phenyl ester (XXII)



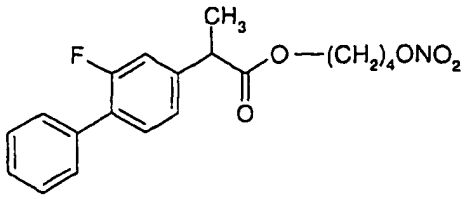
(XXII)

- trans 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid-, 6-(nitrooxymethyl)-2-pyridinylmethylester hydrochloride (XXIII)



(XXIII)

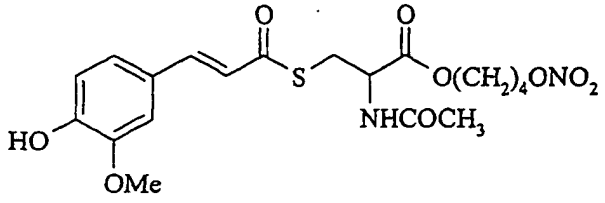
When the precursor drug is flurbiprofen and b0 = 0, c0 = 1, Y of C is R'O, the following compound can be mentioned: 2-fluoro-alpha-methyl-[1,1'-biphenyl]-4-acetic acid (nitrooxy butyl)ester (XXX):



(XXX)

Other preferred compounds of the invention are those wherein in formula (I) the precursor drug of R has formula (IVA), b0= 1 and c0 = 1 and C is Y_0 or Y_{Ar}, in particular the following compounds can be mentioned:

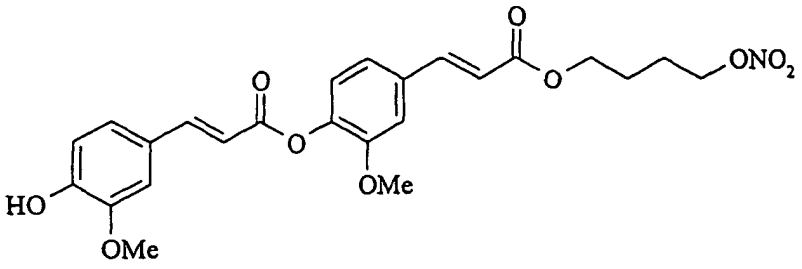
- (S)-N-acetylcysteine-4-(nitrooxy)butylester-, trans-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate (XXIV)



(XXIV)

The preferred compound is the following:

- trans-3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid-, 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxo-1-propenyl]phenylester (XXV)



(XXV)

The nitrooxyderivatives of the compound of formula (IVA) are novel.

These compounds can be prepared by using for example the

following synthesis schemes.

The reactions are carried out by well known methods of the prior art, which allow to obtain bonds among the precursor drug, the precursor compound of B and the bivalent radical C as above defined.

1a. When the drug has the general formula R-COOH, the functional group of the precursor compound of B which binds itself to the carboxylic function of the drug has formula XZ, X being as above defined and Z = H, the reactions which take place depend on the nature of the second reactive group present in the precursor compound of B.

1a.1 When the second reactive group present in the precursor compound of B is a carboxylic group, the general synthesis scheme expects the initial formation of the acid halide R-COHal (Hal = Cl, Br) and the subsequent reaction with the HX group of the precursor compound of B:

$$RCOOH \dashrightarrow RCOHal + H\text{-}X\text{-}X_2\text{-}COOH \dashrightarrow R\text{-}T_1\text{-}T_B\text{-}X_2\text{-}COOH \qquad (IA.1)$$

X_2, T_1, T_B being as above defined.

When in the two compounds under reaction other functional groups COOH and/or HX are present, they must be protected before the reaction according to the methods known in the prior art; for example as described in the volume by Th. W. Greene: "Protective groups in organic synthesis", Harward University Press, 1980.

The RCOHal acylhalide is prepared according to the methods known in the prior art, for example by thionyl or oxalyl chloride, halides of P^{III} or P^{V} in solvents

inert under the reaction conditions, such as for example toluene, chloroform, DMF, etc.

In particular, if the HX group of the precursor compound of B is NH_2, or OH or SH, the precursor drug of formula R-COOH is first converted into the corresponding acyl halide RCOHal, as above mentioned, and then reacted with the HX group of the precursor compound of B in the presence of an organic base, such as triethylamine, pyridine, etc. using a solvent inert under the reaction conditions as toluene, tetrahydrofuran, etc. at a temperature in the range 0°C-25°C.

Alternatively to the previous synthesis, the precursor drug of formula R-COOH can be treated with an agent activating the carboxyl group selected from N,N'-carbonyldiimidazol (CDI), N-hydroxybenzotriazol and dicyclohexylcarbodiimide in solvent such as for example DMF, THF, chloroform etc. at a temperature in the range from -5°C to 50°C and the obtained compound reacted in situ with the reactive function of the precursor compound of B to obtain the compound of formula (IA.1).

1a.2 When the precursor compound of B contains two functional groups XZ, equal to or different from each other, X being as above defined and Z = H, the precursor drug having formula R-COOH is first treated with an agent activating the carboxyl groups, as described in 1a.1, and then with the precursor compound of B, after having protected one of the two reactive groups HX, for example with an acetyl group or tert-butyloxycarbonyl, restoring the initial function at the end of the synthesis. The scheme is the following:

$$RCOOH \xrightarrow{CDI,\ HX\text{-}X_2\text{-}X\text{-}G} R\text{-}T_1\text{-}T_B\text{-}X_2\text{-}X\text{-}G \longrightarrow R\text{-}T_1\text{-}T_B\text{-}X_2\text{-}XH \quad (IA.2)$$

wherein X, T_1, T_B, X_2 are as above defined and G is a protective group of the HX function.

2. Synthesis of the nitrooxyderivative

2a.1 When the compound obtained at the end of the previous step 1a. has formula (IA.1), the acid can be converted into the corresponding sodic salt and then one can follow the methods known in the prior art for preparing the final compound, for example according to one of the following synthesis schemes:

A.) $$R\text{-}T_1\text{-}T_B\text{-}X_2\text{-}COONa + R_4\text{-}X_1\text{-}R_3 \longrightarrow$$

$$R\text{-}T_1\text{-}T_B\text{-}X_2\text{-}T_{BI}\text{-}T_C\text{-}X_{1A}\text{-}R_3 \quad (1A.1b) \xrightarrow{AgNO_3} R\text{-}T_1\text{-}T_B\text{-}X_2\text{-}T_{BI}\text{-}T_C\text{-}Y\text{-}NO_2$$

wherein T_1, T_B, X_2, T_{BI}, T_C are as above, R_4 is selected from Cl, Br, Y is as above defined, X_{1A} is the radical Y without the oxygen atom, R_3 is Cl, Br, Iodine, OH. When R_3 = OH the compound of formula (1A.1b) is subjected to halogenation, for example with PBr_3, PCl_5, $SOCl_2$, PPh_3 + I_2, and then reacted with $AgNO_3$ in organic solvent such as acetonitrile, tetrahydrofuran. When R_3 is Cl, Br, Iodine, the compound of formula (1A.1b) is directly reacted with $AgNO_3$ as above mentioned.

B.) $$R\text{-}T_1\text{-}T_B\text{-}X_2\text{-}COONa + Hal\text{-}Y\text{-}NO_2 \longrightarrow R\text{-}T_1\text{-}T_B\text{-}X_2\text{-}T_{BI}\text{-}T_C\text{-}Y\text{-}NO_2$$

C.)

$R\text{-}T_1\text{-}T_B\text{-}X_2\text{-}COCl + R_5\text{-}X_1\text{-}R_3 \rightarrow R\text{-}T_1\text{-}T_B\text{-}X_2\text{-}T_{BI}\text{-}T_C\text{-}X_{1A}\text{-}R_3$ (1A.1c)

$AgNO_3$

$R\text{-}T_1\text{-}T_B\text{-}X_2\text{-}T_{BI}\text{-}T_C\text{-}X_{1A}\text{-}R_3 \dashrightarrow R\text{-}T_1\text{-}T_B\text{-}X_2\text{-}T_{BI}\text{-}T_C\text{-}Y\text{-}NO_2$

wherein R_5 = OH or NHR_{1C}, R_{1C}, R_3 and the other symbols being as above defined.

The above shown reactions are well known in the prior art. See for example EP 670825 B1, EP 722434 B1 and EP 759899 B1 in the name of the Applicant.

When X_{1A} is a linear C_4 alkyl, the corresponding acid $R\text{-}T_1\text{-}T_B\text{-}X_2\text{-}COOH$ is reacted with triphenylphosphine in the presence of a halogenating agent such as CBr_4 or N-bromosuccinimide in tetrahydrofuran obtaining the compound (1A.1c) wherein R_3 = Br.

2a.2 When the compound obtained at the end of the previous step 1a. has formula (IA.2), the corresponding nitrooxyderivative is obtained by treating a halogen-carboxylic acid of formula $Hal\text{-}X_{1A}\text{-}COOH$, X_{1A} being as above, first with an agent activating the carboxyl group as described in 1A.1, and then with the compound of formula (IA.2), obtaining a halogen derivative, which is isolated and then dissolved in organic solvent (ref. paragraph 2a.1), and treated with silver nitrate. The global reaction scheme is the following:

1) CDI, 2) $R\text{-}T_1\text{-}T_B\text{-}X_2\text{-}XH$

$Hal\text{-}X_{1A}\text{-}COOH \dashrightarrow$

$AgNO_3$

$R\text{-}T_1\text{-}T_B\text{-}X_2\text{-}T_{BI}\text{-}T_C\text{-}X_{1A}\text{-}Hal \dashrightarrow$

$R-T_1-T_B-X_2-T_{BI}-T_C-Y-NO_2$

wherein T_1, T_B, X_2, T_{BI}, T_C, Y are as above.

Alternatively, the halide $Hal-X_1-COCl$ can be used, wherein Hal is preferably bromine, which is reacted with the compound of formula (IA.2).

1b. When the drug precursor has the reactive function HX, wherein X is as above defined, instead of a carboxylic group, the two functional groups present in the precursor compound of B can be the following:

1b.1 A carboxylic group, which reacts with the HX function of the drug precursor, and a group HX, the latter reactive group of the precursor compound of B being equal to or different from the functional group of the drug precursor. The formula of the precursor compound of B is of the $H-X-X_2-COOH$ type, wherein X and X_2 are as above.

The function H-X- of the precursor compound of B is protected according to the known methods in the prior art and the carboxyl group is reacted, as above indicated, according to the following scheme:

$$H-X-X_2-COOH \dashrightarrow G-X-X_2-COOH + R-XH \dashrightarrow$$

$$R-T_1-T_B-X_2-X-G \dashrightarrow R-T_1-T_B-X_2-X-H \quad (1B.1)$$

At the end of the reaction the HX function of the precursor compound of B is restored.

1b.2 When the precursor compound of B contains two carboxylic groups, it is treated with an equimolar amount of an agent activating the carboxyl group under the conditions previously described in 1a.1, and then reacted with the reactive function HX of the drug precursor molecule. Possible other reactive functions of HX type present in the two compounds must be protected as previously

indicated. Lastly a compound of formula $R-T_1-T_B-X_2-COOH$ (1B.2) is obtained.

2b. Synthesis of the nitrooxyderivative.

2b.1 To obtain the final nitrooxyderivative starting from the compound of formula $R-T_1-T_B-X_2-X-H$ (1B.1), obtained at the end of the synthesis described in 1b.1, the compound (1B.1) is reacted with a halogenacid of formula $Hal-X_{1A}-COOH$ which has been treated as previously described in paragraph 1a.1, or with the corresponding halogen acid chloride. The resulting compound is dissolved in organic solvent, for example acetonitrile or tetrahydrofuran, and reacted with silver nitrate.

2b.2 To obtain the final nitrooxyderivative starting from the compound of formula $R-T_1-T_B-X_2-COOH$ (1B.2), obtained at the end of the synthesis described in 1b.2, the acid is transformed into the corresponding sodic salt, it is reacted with a compound $R_4-X_{1A}-R_3$, previously defined in the reaction scheme A. of paragraph 2a.1, obtaining according to the same procedure therein mentioned the final nitrooxyderivative. Alternatively, when X_{1A} is a linear C_4 alkyl, the acid (1B.2) is reacted with triphenylphosphine in the presence of a halogenating agent such as CBr_4 or N-bromosuccinimide in tetrahydrofuran and the resulting compound dissolved in organic solvent, for example acetonitrile, tetrahydrofuran, is reacted with silver nitrate.

2b.3 Alternatively to the synthesis procedure according to 1b.1 and 2b.1, it is possible to react in a first step the HX- function of the precursor compound of B $HX-X_2-COOH$ with the acyl chloride of a halogenacid of formula

Hal-X_{1A}-COCl, wherein Hal is preferably Br, and subsequently the carboxylic function of the so obtained compound with the drug precursor R-HX. In the third and last step the -Hal group is substituited with -ONO_2 according to the process described in 2b.1. The reaction scheme is the following:

$$HX\text{-}X_2\text{-}COOH + Hal\text{-}X_{1A}\text{-}COCl \xrightarrow{R\text{-}XH} Hal\text{-}X_{1A}\text{-}T_C\text{-}T_{BI}\text{-}X_2\text{-}COOH$$

$$Hal\text{-}X_1\text{-}T_C\text{-}T_{BI}\text{-}X_2\text{-}COOH\ (2B.3) \longrightarrow Hal\text{-}X_{1A}\text{-}T_C\text{-}T_{BI}\text{-}X_2\text{-}T_B\text{-}T_1\text{-}R$$

$$Hal\text{-}X_{1A}\text{-}T_C\text{-}T_{BI}\text{-}X_2\text{-}T_B\text{-}T_1\text{-}R \xrightarrow{AgNO_3} O_2N\text{-}Y\text{-}T_C\text{-}T_{BI}\text{-}X_2\text{-}T_B\text{-}T_1\text{-}R$$

wherein T_C, T_{BI}, T_B, T_1, X_2, X_{1A}, Y are as above defined.

In the previous scheme the nitration can be alternatively carried out on the acid compound of formula (2B.3).

When the compounds of formula (I) usable in the present invention have one or more chiral centres, they can be in racemic form or as mixtures of diastereoisomers, as single enantiomers or single diastereoisomers; if they show geometric asymmetry, the compounds in the cis or trans form can be used.

When in the molecule of the compounds of formula (I) a salifiable functional group is present, for example an aminic or heterocyclic nitrogen, it is possible to use the corresponding salts of the above mentioned compounds, obtainable by reaction in organic solvent such as for example acetonitrile, tetrahydrofuran, with an equimolar amount of the corresponding organic or inorganic acid.

Examples of usable organic acids are the following: oxalic, tartaric, maleic, succinic, citric acid.

Examples of usable inorganic acids are the following: nitric, hydrochloric, sulphuric, phosphoric acid. Nitric and hydrocloric acids are preferred.

It has been found that the nitrooxyderivatives used in the present invention are able to prevent the deposition of the amyloid plaque, with a higher efficacy than that of the products of the prior art. Their action mechanism is not clear yet since it has been found that they do not exert any inhibitory effect on the inflammatory process affecting the microglia. The invention results are still more surprising if one considers that the compounds used in the present invention are able to systemically release nitric oxide, which in the Alzheimer pathology is known as one of the pro-inflammatory mediators which are released further to the activation of the microglia. It was not predictable that the use of these compounds in the prophylaxis and therapy of the Alzheimer disease resulted effective.

Furthermore the compounds used in the present invention show very good tolerability, even after a long treatment. Due to the high efficacy and tolerability, they can be used also in very advanced pathology conditions.

The aforesaid compounds are formulated in the corresponding pharmaceutical compositions for parenteral, oral use according to the techniques well known in the art, together with the usual excipients; see for example the volume "Remington's Pharmaceutical Sciences 15a Ed.".

The amount on a molar basis of the active principle in said formulations is the same or lower than the maximum

posology indicated for the precursor drugs. Also higher doses can be used considering their very good tolerability. The daily doses of the precursor drugs can be found in the publications of the field, such as for example in "Physician's Desk reference".

Optionally, the use according to the present invention can be carried out by using the compounds of formula (I) in combination with one or more beta-amyloid based vaccines or antibodies against amyloid-β-peptides (Aβ).

The vaccines are prepared by known methods, for example as described in the publication of Schenk et al. (1999) "Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse" Nature 400(6740):173-7 and F. Bard, Nature vol. 6, 8, 916-919, 2000. In this case it is possible to reduce the administered vaccine amount with consequent reduction of the vaccine side effects of immunological nature as above indicated.

The following Examples illustrate the invention and are not limitative of its scope.

EXAMPLE 1

Synthesis of (S)- N-acetylcysteine-4-(nitrooxy)butylester, -(S)-6-methoxy- alpha-methyl-2-naphthalenacetate of formula (XV)

a) Synthesis of (S,)-N-acetyl-S-(6-methoxy-α-methyl-2-naphthalen acetyl)cysteine

To a solution of 6-methoxy-α-methyl-2-naphtalenacetic acid (10 g, 43.4 mmoles) in chloroform (100 ml) and N,N-dimethylformamide (6 ml) 1,1'-carbonyldiimidazol (CDI) (7.04 g, 43.4 mmoles) is added. After 15 minutes the obtained solution is treated with (S)-N-acetylcysteine (7.08 g, 43.4 mmoles) and it is left at room temperature for 12 hours. The reaction mixture is washed with HCl 5%, then with water and lastly with brine. The organic phase is anhydrified with sodium sulphate and then evaporated at reduced pressure. The obtaind residue is purified by chromatography on silica gel eluting with ethyl acetate. 11.66 g of the expected product are obtained in the form of a white solid m.p. 122°C-126°C.
^{1}H-NMR ($CDCl_3$): 7.71-7.65 (3H, m), 7.34 (1H, dd), 7.16-7.09 (2H, m), 6.36 (1H, d), 4.67 (1H, m), 4.00 (1H, q), 3.90 (3H, s) 3.32 (2H, t), 1.84 (3H, s), 1.59 (3H, d).

b) Synthesis of (S)-N-acetyl-cysteine 4-(bromobutyl) ester-S-6-methoxy-α-methyl-2-naphthalenacetate

To a solution of the compound sinthetized in the previous step (11.3 g, 30.1 mmoles) in tetrahydrofuran (200 ml), triphenylphosphine (23.7 g, 90.3 mmoles) and carbon tetrabromide (28.85 g, 90.3 mmoles) are added. The reaction mixture is left under stirring for 24 hours at room temperature. The solvent is removed by evaporation at reduced pressure. The obtained

crude product is purified by chromatography on silica gel eluting with n-hexane/ethyl acetate 7/3. 4 g of the ester are obtained in the form of a white solid having m.p. 67°C-71°C.

c) Synthesis of (S)- N-acetylcysteine-4-(nitrooxy)butylester, -(S)-6-methoxy- alpha-methyl-2-naphthalenacetate

To a solution of the the ester obtained at the end of the previous step (1 g, 1.96 mmoles) in acetonitrile (20 ml) silver nitrate (0.66 g, 3.92 mmoles) is added. The reaction mixture is heated for 7 hours under reflux sheltered from light. The formed salt is removed by filtration and the solution is evaporated at reduced pressure. The obtained residue is purified by chromatography on silica gel eluting with n-hexane/ethyl acetate 7/3. 0.47 g of the final product are obtained in the form of a white solid having m.p. 56°C-59°C.

^{1}H-NMR ($CDCl_3$) : 7.80-7.68 (3H, m), 7.37(1H, d), 7.20-7.13 (2H, m), 6.12 (1H, d) 4.40 (2H, dd), 4.26 (1H, m), 4.15-3.87 (3H, m), 3.92 (3H, s), 3.33 (2H, d), 1.86 (3H, d), 1.74-1.67 (4H, m), 1.61 (3H, d).

Elementary analysis:

Calculated	C: 56.08%	H: 5.73%	N: 5.71%	S: 6.51%
Found	C: 55.99%	H: 5.68%	N: 5.60%	S: 6.35%

EXAMPLE 2

Synthesis of (S)-N-acetylcysteine-4-(nitrooxy)butylester, -alpha-methyl-4-(2-methylpropyl)benzeneacetate having formula (XVIII) (NO ibuprofen)

a) Synthesis of (S)-N-acetyl-S-[α-methyl[4-(2-methylpropyl) benzenelacetyl]cysteine

To a solution of α-methyl[4-(2-methylpropyl)benzene] acetic acid (10 g, 48.48 mmoles) in chloroform (100 ml) and N,N-

dimethylformamide (6 ml), 1,1'-carbonyldiimidazol (7.86 g, 48.48 mmoles) is added. After 1 hour the obtained solution is treated with (S)-N-acetylcysteine (7.91 g, 48.47 mmoles) and it is left at room temperature for 24 hours. The reaction mixture is washed with HCl 5%, then with water and lastly with brine. The organic phase is anhydrified with sodium sulphate and then evaporated at reduced pressure. The obtained residue is purified by chromatography on silica gel eluting with ethyl acetate. 13.3 g of the expected product are obtained in an oil form.

^{1}H-NMR ($CDCl_3$): 10.17 (1H, s) 7.13 (2H, d) 6.54 (1H, d), 4.76 (1H, m), 3.93 (1H, q), 3.42-3.30 (2H, m), 2.49 (2H, d), 1.85-1.83 (4H, m), 1.55 (3H, d), 0.93 (6H, d).

b) Synthesis of (S)-N-acetylcysteine-4-(bromo)butylester, -alpha-methyl-4-(2-methylpropyl)benzeneacetate

To a solution of the compound synthetized in the previous step (12.8 g, 36.4 mmoles) in tetrahydrofuran (100 ml) triphenylphosphine (28.65 g, 109.23 mmoles) and carbon tetrabromide (36.23 g, 109.23 mmoles) are added. The reaction mixture is left under stirring for 48 hours at room temperature. The solvent is removed by evaporation at reduced pressure. The obtained crude product is purified by chromatography on silica gel eluting with cyclohexane/ethyl acetate 1/1. 5.79 g of the ester are obtained in an oil form.

c) Synthesis of (S)-N-acetylcysteine-4-(nitrooxy)butylester, -alpha-methyl-4-(2-methylpropyl)benzeneacetate

To a solution of the ester obtained at the end of the previous step (5.5 g, 11.3 mmoles) in acetonitrile (100 ml) silver nitrate (2.69 g, 15.8 mmoles) is added. The reaction mixture is heated for 24 hours under reflux sheltered from

light. The formed salt is removed by filtration and the solution is evaporated at reduced pressure. The obtained residue is purified by chromatography on silica gel eluting with cyclohexane/ethyl acetate 7/3. 1.18 g of the expected product are obtained in an oil form.

^{1}H-NMR ($CDCl_3$): 7.27-7.09 (4H, m), 6.19 (1H, d), 4.75 (1H, m), 4.47 (2H, t), 4.15-4.02 (2H, m), 3.86 (1H, q), 3.31 (2H, d), 2.44 (2H, d), 1.89 (3H, d), 1.86-1.76 (5H, m), 1.51 (3H, d), 0.89 (6H, d).

Elementary analysis:

Calculated	C: 56.39%	H: 6.88%	N: 6.00%	S: 6.84%
Found	C: 56.22%	H: 6.79%	N: 5.88%	S: 6.92%

EXAMPLE 3

Synthesis of (S)- N-acetylcysteine-4-(nitrooxy)butylester, 1-(4-chloro benzoyl)-methoxy-2-methyl-1H-indol-3-acetate of formula (XVII)

a) Synthesis of (S)-N-acetyl-S-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-acetyl]cysteine

To a solution of 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-acetic acid (10 g, 28.00 mmoles) in chloroform (100 ml) and N,N-dimethylformamide (2 ml), 1,1'-carbonyldiimidazole (4.53 g, 28.00 mmoles) is added. After 1 hour the obtained solution is treated with (S)-N-acetylcysteine (4.56 g, 28.00 mmoles) and it is left at room temperature for 24 hours. The reaction mixture is washed with HCl 5%, then with water and lastly with brine. The organic phase is anhydrified with sodium sulphate and then evaporated at reduced pressure. The obtained residue is purified by chromatography on silica gel eluting with ethyl acetate. 7.79 g of the expected product are obtained in the form of a yellow solid having m.p. 129°C.

^{1}H-NMR (DMSO-d_6): 12.90 (1H, s), 8.21 (1H, d), 7.69-7.64 (4H, m), 7.06 (1H, d), 6.96 (1H, d), 6.73 (1H, dd), 4.33 (1H, m), 4.02 (2H, s), 3.77 (3H, s), 3.33-2.96 (2H, m), 2.22 (3H, s), 1.78 (3H, s).

b) Synthesis of (S)- N-acetylcysteine-4-(bromo)butylester, 1-(4-chloro benzoyl)-methoxy-2-methyl-1H-indol-3-acetate

To a solution of the compound obtained in the previous step (3.09 g, 6.14 mmoles) in N,N dimethylformamide (50 ml), sodium ethylate (0.42 g, 6.14 mmoles) and, after 30 minutes, 1,4-dibromobutane (2.18 ml, 18.00 mmoli) dissolved in 25 ml of N, N dimethylformamide, are added. The reaction mixture is left under stirring for 20 hours at room temperature, then it is diluted with ethyl ether and washed with water. After the organic phase has been anhydrified with sodium sulphate the solvent is removed by evaporation at reduced pressure. The obtained crude product is purified by chromatography on silica gel eluting with cyclohexane/ethyl acete 1/1. 1.7 g of the ester are obtained in the form of a yellow solid having m.p. 130°C-134°C.

c) Synthesis of (S)- N-acetylcysteine-4-(nitrooxy)butylester, 1-(4-chlorobenzoyl)-methoxy-2-methyl-1H-indole-3-acetate

To a solution of the ester obtained at the end of the previous step (1.6 g, 2.5 mmoles) in acetonitrile (30 ml) silver nitrate (0.6 g, 3.51 mmoles) is added. The reaction mixture is heated for 8 hours under reflux sheltered from light. The formed salt is removed by filtration and the solution is evaporated at reduced pressure. The obtained residue is purified by chromatography on silica gel eluting with cyclohexane/ethyl acetate 4/6. 1.2 g of the final product are obtained in an oil form.

^{1}H-NMR ($CDCl_3$): 7.66 (2H, d), 7.48 (2H, d), 6.90 (2H, m), 6.68 (1H, m), 6.14 (1H, d), 4.77 (1H, m), 4.43 (2H, t), 4.08 (2H, m), 3.87 (2H, s), 3.83 (3H, s), 3.34 (2H, d), 2.38 (3H, s), 1.90 (3H, s), 1.78-1.70 (4H, m).

Elementary analysis:

Calculated C: 54.24% H: 4.88% N: 6.80% S: 5.17% Cl: 5.72%

Found C: 54.32% H: 4.93% N: 6.91% S: 5.13% Cl: 5.84%

EXAMPLE 4

Synthesis of (S)- N-acetylcysteine-4-(nitrooxy)butylester, 2-fluoro-alpha-methyl-[1,1-biphenyl]-4-acetate of formula (XVI)

The compound is synthetized according to the procedure described in Example 1. The substance appears as an oil. Yield: 26%.

^{1}H-NMR ($CDCl_3$): 7.41-7.38 (6H, m), 7.10 (2H, m), 6.22 (1H, d), 4.78 (1H, m), 4.46 (2H, t), 4.13 (2H, t), 3.92 (1H, q), 3.36 (2H, d), 1.93 (3H, d), 1.76 (4H, d), 1.55 (3H, d).

Elementary analysis:

Calculated C: 56.91% H: 5.37% N: 5.55% S: 6.33% F: 3.75%

Found C: 56.99% H: 5.41% N: 5.66% S: 6.41% F: 3.83%

EXAMPLE 5

Synthesis of alpha-methyl-4-(2-methylpropyl)benzeneacetic acid, 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxo-1-propenyl]phenylester of formula (XIII)

a) Synthesis of the trans-3-[4-[α-methyl-[4-(-2-methylpropyl) benzene]acetyloxy]-3-methoxyphenyl] -2-propenoic acid

To a solution of α-methyl-[4-(2-methylpropyl)benzene]acetic acid (5.03 g, 24.4 mmoles) in tetrahydrofuran (100 ml) and N,N-dimethylformamide (5 ml) 1,1-carbonyldiimidazol (4.25 g, 24.8 mmoles) is added. After 1 hour the obtained solution is treated with ferulic acid (4.90 g, 25 mmoles), sodium ethy-

late (89 mg) is added and the solution is left at room temperature under stirring for 12 hours. The reaction mixture is washed with HCl 5%, then with water and lastly with brine. The organic phase is anhydrified with sodium sulphate and evaporated at reduced pressure.

The obtained residue is purified by chromatography on silica gel eluting with ethyl acetate/n-hexane 7/3. 5.1 g of the expected product are obtained as a white solid having m.p. 131°C-137°C.

^{1}H-NMR ($CDCl_3$): 7.72 (1H, d), 7.32 (2H, dd), 7.26 (1H, m), 7.16-7.07 (4H, m), 6.98 (1H, d), 6.37 (1H, d), 3.99 (1H, q), 3.73 (3H, s), 2.47 (2H, d), 1.88 (1H, m), 1.63 (3H, d), 0.92 (6H, d).

b) synthesis of benzenacetic acid, alpha-methyl-4-(2-methylpropyl), 2-methoxy-4-[(1E)-3-[4-(bromo)butoxy]-3-oxo-1-propenyl]phenylester

To a solution of the compound synthetized in the previous step (5.33 g, 14 mmoles) in N,N-dimethylformamide (130 ml) sodium ethylate (1.2 g, 16 mmoles) is added, under stirring. After 1 hour to the obtained mixture 1,4-dibromobutane (10 g, 46 mmoles) is added and it is let react at room temperature for 12 hours. The reaction mixture is washed with HCl 5%, then with water and lastly with brine; the organic phase is anhydrified with sodium sulphate and evaporated at reduced pressure. The obtained residue is purified by chromatography on silica gel eluting with n-hexane/ethyl acetate 8/2. 4.46 g of the expected product are obtained.

c) Synthesis of benzenacetic acid, alpha-methyl-4-(2-methyl-propyl), 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxo-1-propenyl]phenylester

To a solution of the compound synthetized in the previous step (4 g, 7.72 mmoles) in acetonitrile (70 ml) silver nitrate (2.58 g, 15 mmoles) is added. The reaction mixture is heated under reflux for 2 hours sheltered from light. At the end the formed salt is removed by filtration and the solution is evaporated at reduced pressure. The recovered residue is purified by chromatography on silica gel eluting with n-hexane/ethyl acetate 8/2. 2.4 g of the expected product are obtained as an oil.

^{1}H-NMR ($CDCl_3$): 7.62 (1H, d), 7.32 (2H, d), 7.15 (2H, d), 7.16-7.05 (2H, m), 6.96 (1H, d), 6.35 (1H, d), 4.51 (2H, t), 4.24 (2H, t), 3.99 (1H, q), 3.74 (3H, s), 2.48 (2H, d), 1.89-1.83 (5H, m), 1.62 (3H, d), 0.92(6H, d).

Elementary analysis:

Calculated	C: 64.91%	H: 6.66%	N: 2.82%
Found	C: 64.83%	H: 6.52%	N: 2.69%

EXAMPLE 6

Synthesis of [1,1'-biphenyl]-4-acetic acid, 2-fluoro-alpha-methyl,2-methoxy-4-[(1E)-3-[4-(nitrooxy)butoxy]-3-oxo-1-propenyl]phenylester of formula (XII) (NO-flurbiprofen)

The compound is synthetized according to the procedure described in Example 5. The global yield of the process is 32%. The substance appears as an amorphous solid.

^{1}H-NMR ($CDCl_3$): 7.40-7.25 (9H, m), 7.07-7.01 (2H, d), 6.98 (1H, m), 6.38 (1H, d), 4.44 (2H, t), 4.46 (2H, t), 4.21 (2H, t), 4.04 (1H, q), 3.73 (3H, s), 1.72 (4H, m), 1.65 (3H, d).

Elementary analysis:

Calculated	C: 64.79%	H: 5.25%	N: 2.62%	F: 3.53%
Found	C: 64.85%	H: 5.31%	N: 2.74%	F: 3.48%

EXAMPLE 7

Evaluation of the effect of ibuprofen and of NO-flurbiprofen (ex. 6) on the reduction of the amyloid deposits (amyloid plaques) (Protein Aß) in the front cerebral cortex and on the activation of the microglia measured in the frontal cerebral cortex.

In this experiment transgenic rats (APPM-PS1M) having human genes and mutated by the protein precursor of the ß-amyloid protein (APP, from which the Aß protein of the amyloid plaques forms by proteolysis) and by the preseniline-1 proteine(PS1) have been used. Mutations in these proteins cause the Alzheimer dementia in some families wherein the disease presents a dominant autosomic character. Rats carriers of the double-transgene APP mutated + PS-1 mutated (APPM/PS1M) represent a very good model for the study of Alzheimer dementia since they develop amyloid plaques and neuroinflammation like those found in the human pathology (Holcomb L. et Al. Nat. Med. 4:97 (1998)).

Three groups each formed by 12 transgenic rats (APPM/PS1M), 7 months old at the beginning of the experiment, received together with the daily diet, ibuprofen and, respectively, NO-flurbiprofen (ref. Ex. 6).

The administered pro die dose of each compound was 60 mg/kg for 5 months. The third group was the control group and received only the daily diet. At the end of the fifth month the animals were sacrificed by intra-peritoneal injection of pentobarbital (100 mg/kg). The brains were removed and the

two hemispheres separated. The hemispheres were first treated for 24 hours with a paraformaldehyde solution (4% by volume), then for a period of 8 hours each, with solutions at increasing concentrations of phosphate buffer of Soreson, respectively at 10%, 20% and 30%. The hemispheres have then been frozen and dissected along an horizontal plane and the sections have been maintained at 4°C in DPBS (Dulbecco phosphate buffer saline) with sodium azide.

The entity of the amyloid deposits has been evaluated on a part of the brain sections obtained by coloration with Congo Red (Sheehan DC, Hrapchak BB, eds. Theory and Practice of Histotechnology, 2nd ed. Mosby, St. Louis (MO), 1980, pp. 177-178; Thompson SW, ed. Selected Histochemical and Histopathological Methods. Charles C. Thomas, Springfield (IL), 1966, pp. 402-405), and on a further part of the brain sections by treatment with polyclonal antibody specific versus peptide Aß, in order to identify by a susequent immunochemical method the Aß deposits.

Another part of the sections obtained from the frontal cortex has been analyzed by immunohistochemical analysis by using antibodies directed against microglial activation indicators: anti-MHC-II, directed against the hystocompatibility complex of type II, anti-CRIII which recognizes the complement receptor 3. Expression of these indicators results increased in the activated microglia. The microglia presence has been quantified by videodensitometric analysis and the positive microglial cells for MHC-II have been counted in various areas of each brain section and the results have been expressed as average number of positive MHC-II cells for tissue section.

The results are reported in Tables 1 and 2.

The results reported in Table 1 show that NO-flurbiprofen induces a significant reduction of the amyloid plaque deposits (Aß deposits) in treated animals with respect to ibuprofen.

The ibuprofen activity results remarkably lower than that of the compounds according to the present invention.

The results reported in Table 2 are expressed as a ratio between the average number of activated microglial cells (positive MCH-II) counted in the cerebral sections of the three groups of animals and the average number of activated microglial cells counted in the cerebral sections of the control group. Said results show that in the group of rats treated with NO-flurbiprofen the number of the microglial cells is about 25 times higher than that of the controls and that in the group treated with ibuprofen the number of the activated cells is about 6 times higher than that of the controls.

EXAMPLE 8

Evaluation of the gastric damages induced by flurbiprofen and ibuprofen with respect to the corresponding nitrooxyderivatives according to the present invention

The gastric tolerability of these drugs has been evaluated in rats (groups of 10 rats each) treated for short or long period, according to conventional protocols, respectively with NO-flurbiprofen as above defined, an equimolar dose of flurbiprofen, or with ibuprofen or with an equimolar dose of NO-ibuprofen.

The administered doses and the obtained results are reported in Table 3 and show that administration of flurbipro-

fen causes gastric damages, while NO-flurbiprofen does not damage the gastric mucosa, and is more tolerated.

EXAMPLE 9

Efficacy of NO-flurbiprofen (ex. 6), of 4-nitrooxy butyl derivative of flurbiprofen (NO-C_4-flurbiprofen), of 4-nitrooxybutyl derivative of aspirin (NO-ASA) and of flurbiprofen in inhibiting the cerebral neurodegeneration in rats treated with LPS

The nitrooxybutyl derivative of aspirin (NO-ASA) and the nitrooxybutyl derivative of flurbiprofen (NO-C_4- flurbiprofen) have been synthetized according to the method reported in the patent application WO 95/30641 in the name of the Applicant.

72 male rats F-344, 3-months old, have been divided in 5 groups, which were chronically infused with LPS (lypopolysaccharide) for 30 days and received once a day for the same period of time, by subcutaneous injection respectively NO-ASA (90 mg/kg equivalent to 302 μmoles/Kg), NO-flurbiprofen (14 μmoles/kg), NO-C_4-flurbiprofen (15 mg/Kg equivalent to 41.5 μmoles/kg), flurbiprofen (3 mg/Kg equivalent to 12.3 μmoles/kg), or only the same volume of solvent (controls). The solvent was formed by a mixture of dimethylsolphoxide/ethanol/castor oil 20/5/75 by volume.

At the end rats were sacrificed by lethal injection with pentobarbital.

Fixation procedure for cerebral tissues

The brain of each rat was perfused in situ at first with a cold saline solution containing 1 U/ml of heparin, then with paraformaldehyde 4% in phosphate buffer 0,1 M (pH 7.4). The brains have been dissected, further on fixed for 1 h in the

same solutions and incubated for one night in a cryoprotective solution containing 20% by weight of sucrose. The frozen tissues were gradually warmed up to -20°C and front serial sections were cut by a cryostat. The sections have been collected in phosphate buffer, then immediately used or otherwise maintained overnight.

The sections were washed and incubated in phosphate buffer and incubated overnight at room temperature with primary antibodies for specific epitoms. The activated microglia was visualized by using the antibody OX-6 directed against the histocompatibility complex of class II (MHC-II). Sections have been incubated for 1 h with the secondary biotinylated antibody (mouse anti-IgG, obtained in horse) and then with avidin conjugated with peroxidase (1 h). Then, the sections were treated for 1-5 min with 0.05% of a solution of 3,3'-diaminobenzidine tetrahydrohydrocloride, which is the peroxidase substratum. The dye was removed by washings with PBS.

The brain sections were then analyzed by microscopy. The number of positive microglial cells for OX-6 in the dentate ring and in the CA1-4 areas of the hippocampus were counted in identical brain sections from each rat.

The results, expressed as inhibition percentage of the neurodegenerative process with respect to the controls, are reported in Table 4 and show that the nitrooxyderivatives NO-flurbiprofen and NO-C_4-flurbiprofen according to the invention are more effective in inhibiting the neurodegenerative process induced by LPS with respect to NO-ASA. Flurbiprofen at the tested dose, that is comparable with that of NO-C_4-flurbiprofen, is ineffective an caused gastric lesions in the animals. The

NO-ASA activity is on the contrary very lower than that of the two previous compounds.

Table 1

Inhibition of the amyloid plaque deposit in transgenic rats (APP+PS1) in consequence of the treatment with ibuprofen and NO-flurbiprofen, respectively. The controls have not received any pharmacological treatment		
Treatment	Inhibition % (histochemical method[1])	Inhibition % (imunochemical method[2])
Controls	0	0
NO-Flurbiprofen	40	45
Ibuprofen	14	27

[1] Coloration with congo red;

[2] Dosage Aß I by imunocytochemical method

Table 2

Immuno-histochemical analysis of the activation of microglial cells in consequence of the treatment with ibuprofen and NO-flurbiprofen, respectively.
The controls have not received any pharmacological treatment.
The results are expressed as ratio between the average number of microglial cells in each group of rats with respect to that of the controls (R_{ATT})

Tretament	R_{ATT}
Controls	1
NO-Flurbiprofen	25
Ibuprofen	6

Table 3

Gastric toxicity of ibuprofen and flurbiprofen with respect to NO-ibuprofen and NO-flurbiprofen respectively

Compounds	Dose (mg/kg)	Gastric Damage Score	Compounds	Dose (mg/kg)	Gastric Damage Score
Ibuprofen	3	1	Flurbiprofen	1	5
	10	1		3	10
	30	3.5		10	10
	100	16.5		30	19
NO-Ibuprofen	6.8	0.5	NO-flurbiprofen	2.2	0
	22.7	0.5		6.6	0
	68	2		22	0
	227	0.5		66	0

Table 4

Comparison among the efficacy of NO-flurbiprofen, of nitrooxybutylderivative of aspirin (NO-ASA) and of nitrooxybutyl derivative of flurbiprofen (NO-C_4-flurbiprofen) in inhibiting the cerebral neurodegeneration in rats treated with LPS.		
Treatment	Dose µmoles/Kg	Neurodegeneration inhibition %
Controls	-	0
NO-ASA	302	27.5
NO-C_4-Flurbiprofen	41.5	71
NO-Flurbiprofen	14	71
Flurbiprofen	12,3	0

CLAIMS

1. Use for the preparation of drugs in the prophylaxis and treatment of the Alzheimer desease of compounds, or salts thereof, having the following general formula:

$$A\text{-}(B)_{b0}\text{-}(C)_{c0}\text{-}NO_2 \qquad (I)$$

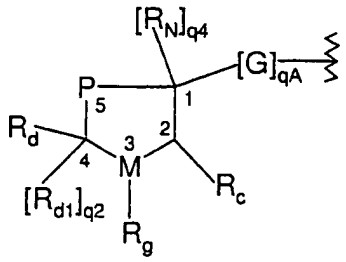
wherein:

c0 is an integer and is 0 or 1, preferably 1;

b0 is an integer and is 0 or 1, with the proviso that c0 and b0 cannot be contemporaneously equal to zero.

A = $R\text{-}T_1\text{-}$, wherein

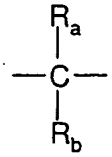
R is the drug radical as defined below:



(IV)

wherein:

G is one of the following groups: -CH=CH-,



(Vg)

M is one carbon or nitrogen atom;

P is the following group: